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14. ABSTRACT

The pathogenic bacterium Bacillus anthracis has become the subject of intense study as a result of its use as a bioterrorism attack in the United States in September and October 2001, Previous studies suggested that B. anthraci8s Ames ancestor, the original Ames fully virulent plasmid containing isolate, was the ideal reference. This study describes the complete genome sequence of that original isolate, derived from a sample kept in cold storage since 1981.

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Genome Announcements

The Complete Genome Sequence of *Bacillus anthracis* Ames "Ancestor"▽

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The pathogenic bacterium *Bacillus anthracis* has become the subject of intense study as a result of its use in a bioterrorism attack in the United States in September and October 2001. Previous studies suggested that *B. anthracis* Ames Ancestor, the original Ames fully virulent plasmid-containing isolate, was the ideal reference. This study describes the complete genome sequence of that original isolate, derived from a sample kept in cold storage since 1981.

We describe the first complete whole-genome sequence (chromosome and plasmids) of a fully virulent isolate of *Bacillus anthracis*, that of the strain known as Ames Ancestor. The *B. anthracis* Ames Ancestor strain was isolated in 1981 from a dead 14-month-old female Beefmaster heifer in Sarita, TX. Tryptose slant cultures of the *B. anthracis* isolate were acquired in February 1981 by researchers at the United States Army Medical Research Institute of Infectious Diseases in Frederick, MD, from the Texas A&M Veterinary Medicine and Diagnostic Laboratory Bacteriology Department. This isolate was shipped in a box with an old address label from Ames, IA, hence the misnomer. To distinguish it from its descendant, this strain is now referred to as *B. anthracis* Ames Ancestor. Previously sequenced *B. anthracis* Ames strains had been obtained from Porton Down, England (Ames Porton), and from the 2001 mailing attack in Florida (A2012 or Ames Florida) (3, 4). While the sequences of *B. anthracis* Ames Porton and Ames Florida are available, they are not ideal as references due to the fact that Ames Porton had been cured of both of its plasmids, pXO1 and pXO2, by heat and novobiocin treatments, respectively (1, 3), and Ames Florida was sequenced only to draft quality (12× coverage). *B. anthracis* Ames Ancestor is the progenitor of all of the Ames strains used as research tools in laboratories around the world; hence, it is an important reference for both basic research and microbial forensics.

The complete genome sequence of *B. anthracis* Ames Ancestor was obtained by whole-genome shotgun sequencing. The genome sequence was assembled by using the Celera Assembler (2). The final sequence had a minimum of two agreeing and high-quality sequence reads for each consensus nucleotide. This completed genome contained an average of 11.5× sequence reads coverage for every nucleotide position of the 5,227,419-bp chromosome, 31.5× for plasmid pXO1 (181,677 bp) and 18.5× for plasmid pXO2 (94,830 bp). The deeper coverage of the plasmids is likely explained by average copy numbers of three (pXO1) and two (pXO2) plasmids per cell. The genome sequence was deposited in GenBank with accession numbers AE017334, AE017336, and AE017335 for the chromosome and plasmids pXO1 and pXO2, respectively. The electropherogram data were deposited in the NCBI Trace Archive, and the sequence assembly is available from the NCBI Assembly Archive (5) under Assembly ID 293. These resources enable studies of sequence variation and detailed comparison of very closely related genomes.

Genome sequence comparison of *B. anthracis* Ames Porton (3) to the draft genome of *B. anthracis* Ames Florida identified polymorphic sites between these two isolates of the Ames strain (4). Interestingly, at each of these polymorphic loci, *B. anthracis* Ames Ancestor was identical to *B. anthracis* Ames

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Florida, indicating that the polymorphisms reported were most likely the result of the plasmid-curing process associated with *B. anthracis* Ames Porton rather than polymorphisms specific to Ames Florida. Having an appropriate high-quality reference sequence was the cornerstone of the scientific investigation into the anthrax mailing attacks of 2001 and was the primary motivation for generating the complete genome sequence of Ames Ancestor.

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REFERENCES

1. **Green, B. D., L. Battisti, T. M. Koehler, C. B. Thorne, and B. E. Ivins.** 1985. Demonstration of a capsule plasmid in *Bacillus anthracis*. Infect. Immun. **49:**291–297.
2. **Myers, E. W., G. G. Sutton, A. L. Delcher, I. M. Dew, D. P. Fasulo, M. J. Flanigan, S. A. Kravitz, C. M. Mobarry, K. H. Reinert, K. A. Remington, E. L. Anson, R. A. Bolanos, H. H. Chou, C. M. Jordan, A. L. Halpern, S. Lonardi, E. M. Beasley, R. C. Brandon, L. Chen, P. J. Dunn, Z. Lai, Y. Liang, D. R. Nusskern, M. Zhan, Q. Zhang, X. Zheng, G. M. Rubin, M. D. Adams, and J. C. Venter.** 2000. A whole-genome assembly of *Drosophila*. Science **287:** 2196–2204.
3. **Read, T. D., S. N. Peterson, N. Tourasse, L. W. Baillie, I. T. Paulsen, K. E. Nelson, H. Tettelin, D. Fouts, J. A. Eisen, S. R. Gill, E. K. Holtzapple, O. A. Økstad, E. Helgason, J. Risltone, M. Wu, J. F. Kolonay, M. J. Beanan, R. J. Dodson, L. M. Brinkac, M. Gwinn, R. J. DeBoy, R. Madpu, S. C. Daugherty, A. S. Durkin, D. H. Haft, W. C. Nelson, J. D. Peterson, M. Pop, H. M. Khouri, D. Raduna, J. L. Benton, Y. Mahamoud, L. Jiang, I. R. Hance, J. F. Weidman, K. J. Berry, R. D. Plaut, A. W. Wolf, K. L. Watkins, W. C. Nierman, A. Hazen, R. Cline, C. Redmond, J. E. Thwaite, O. White, S. L. Salzberg, B. Thomason, A. M. Friedlander, T. M. Koehler, P. C. Hanna, A.-B. Kolstø, and C. M. Fraser.** 2003. The genome sequence of *Bacillus anthracis* Ames and comparison to closely related bacteria. Nature **423:**81–86.
4. **Read, T. D., S. L. Salzberg, M. Pop, M. Shumway, L. Umayam, L. Jiang, E. Holtzapple, J. D. Busch, K. L. Smith, J. M. Schupp, D. Solomon, P. Keim, and C. M. Fraser.** 2002. Comparative genome sequencing for discovery of novel polymorphisms in *Bacillus anthracis*. Science **296:**2028–2033.
5. **Salzberg, S. L., D. Church, M. DiCuccio, E. Yaschenko, and J. Ostell.** 2004. The genome Assembly Archive: a new public resource. PLoS Biol. **2:**E285.